Detection of Desmin in Formalin-Fixed, Paraffin Embedded Mouse Tissue

Reagents:
1X Automation Buffer
3% Hydrogen Peroxide
Antibody Diluent
DAB Chromagen
Hematoxylin

Antibody Information:

Kit : Vector M.O.M. Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog: PK-2200
*The Vector M.O.M. Kit contains solutions needed to make the block, secondary and label antibodies.

Avidin Biotin Blocking Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #SP-2001

Primary Antibody: Monoclonal Mouse anti-Human Desmin
Dako Corporation
Carpinteria CA 93013
www.dakousa.com
1-800-235-5763
Ref No. M0760

Negative control serum: Normal Mouse Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #015-000-001

Staining Procedure

Positive Control Tissue: Smooth and striated muscle cells of the heart
Stain localization: Cytoplasmic

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Automation Buffer	2 times	5 minutes

1. Quench endogenous peroxidase by placing slides in 3% hydrogen peroxide for 15 minutes.

2. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

3. Unmasking Techniques Using the Microwave Oven
   Place a full rack of slides in a Tissue Tek™ container containing 250ml of retrieval solution.
   Microwave for 5 minutes at level 5.
   Cool for 1 minute (Add retrieval solution to the container, if necessary).
   Microwave again for 5 minutes at level 5. Temperature _____
   Remove the slides from the microwave oven and cool 20 minutes at room temperature.
   Rinse slides in distilled water for 2 minutes. Repeat twice.

4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

5. Incubate sections for 1 HOUR in M.O.M. specific IgG blocking reagent
   Made via 2.5 mls 1x PBS plus 2 drops of Mouse IgG blocking reagent
   Kit Lot#_____ Exp Date_____New Kit: yes / no

6. Apply Avidin/Biotin block
   Lot#_____ Exp Date_____ New Kit: yes / no
   Apply avidin block - 15 min at RT.
   Quick rinse in 1X AB.
   Apply biotin block - 15 min at RT.
   Wipe excess block

   DO NOT RINSE SECTIONS WITH BUFFER.

   Prepare Vector M.O.M .diluent: 600ul of protein concentrate stock in 7.5 mls of 1X PBS. Make primary secondary, and label antibody dilution in Vector M.O.M. diluent.

7. Apply primary antibody (Desmin) at a 1:250 dilutions and incubate for one hour:
   Lot#_____ Exp Date_____

   For negative control slides, normalize the protein concentration of normal mouse serum to the protein concentration of the primary antibody (Desmin) and use this to make a 1:250 dilution. Apply to slides and incubate for one hour.
   Lot#_____ Reconstituted Date_____

8. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

9. Apply M.O.M. biotinylated anti-mouse IgG and incubate for 10 minutes
   Made via 10ul of antibody in 2.5mls of Vector M.O.M. diluent.

10. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

11. Apply Vector Elite label for 5 minutes. (Prepare 30 minutes before use)
    Made via 2 drops of Reagent A plus 2 drops of Reagent B in 2.5mls M.O.M. diluent

12. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

13. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.
    (Add 1 drop of DAB per ml of substrate)
    Lot#_____ Exp. Date_____ New Kit: yes / no

14. Rinse in tap water 3 minutes.

15. Counterstain with Modified Harris Hematoxylin for 20 seconds.

16. Rinse in tap water until water is clear.

17. Gently agitate slides in 1X Automation Buffer until blue.

18. Dehydrate through the following solutions.

95% Ethanol	1 change	3 minutes
100% Ethanol	3 changes	3 minutes
Xylene	2 changes	5 minutes

19. Coverslip

Updated 08/18/06